Acid Phosphatase Reagent

Acid Phosphatase Reagent

Order No.	Description
R85303	20 × 3 mL
R85128	20 × 6.5 mL

INTENDED USE

This set of reagents is intended for the quantitative in vitro kinetic determination of acid phosphatase activity in serum.

TEST SUMMARY

Numerous phosphate compounds have been proposed as substrates for measuring acid phosphatase activity, such as phenylphosphate, α-glycerolphosphate, p-nitrophenylphosphate, phenolphthalein phosphate, thymolphthalein phosphate. Alpha-naphthylphosphate was proposed by Babson et al. (1) as a specific substrate for prostatic acid phosphatase. Amador et al. (2) demonstrated, however, that this compound can be hydrolyzed by enzymes derived from other tissues. The α-naphthol liberated by the action of acid phosphatase can be coupled with diazotized 2-amino-5-chlorotoluene (Fast Red TR) to form a diazo dye in a reaction first described by Hillman (3). This dye has a strong absorbance at 405 nm, and the increase in absorbance is proportional to the level of acid phosphatase in the sample. This method of assay was critically evaluated by Fabiny-Byrd and Ertinghausen (4). The following reactions are involved:

$$\alpha\text{-napthylphosphate} + H_2O \xrightarrow[\text{phosphatase}]{\text{acid}} \alpha\text{-naphthol} + \pi$$

$$\alpha\text{-napthol} + \text{Fast Red TR} \longrightarrow \text{Diazo Dye}$$

L-Tartrate inhibits prostatic acid phosphatase but does not interfere with the formation of the color. Thus, if the assay is performed in the presence and in the absence of L-Tartrate, the difference between the results of the two parallel assays gives the level of prostatic acid phosphatase in serum.

Since acid phosphatase is unstable at the pH of the serum, an acetate buffer is provided to acidify the sample and stabilize the enzyme (5).

REAGENTS COMPOSITION

We provide 3 separate reagents for this assay.

Components		Approx. Concentration
1.	Acid Phosphatase Reagent	
	α-naphthylphosphate	3 mmol/L
	Fast Red TR	1 mmol/L
2.	L-Tartrate Reagent	
	Sodium L-Tartrate	2 mol/L
3.	Acetate Buffer	

The reagents contain, in addition, nonreactive stabilizers and fillers.

REAGENTS PREPARATION

Dissolve the contents of each vial of Acid Phosphatase Reagent in the volume of distilled or deionized water specified on the vial label. Store in the refrigerator at 2–8 °C.

Dissolve the contents of each vial of L-Tartrate Reagent with 5 mL of distilled or deionized water. Store in refrigerator at 2–8 °C. If crystallization of components occurs, warm at moderate temperature (40–50 °C) until dissolved. Use the Acetate Buffer solution as provided.

REAGENT STORAGE AND STABILITY

The reagents in the unopened vials are stable until the expiration date on the label when stored in the refrigerator at 2–8 °C.

The Acid Phosphatase Reagent, after reconstitution, is stable for 1 day at room temperature (22–28 °C) and for 7 days in the refrigerator (2–8 °C).

The reconstituted L-Tartrate Reagent and Acetate Buffer are stable in the refrigerator at 2–8 °C until the expiration date on the label.

If the freshly reconstituted Acid Phosphatase Reagent has an absorbance of over 0.300 at 405 nm when measured against a blank of water, this indicates deterioration; do not use. The L-Tartrate solution may precipitate in the refrigerator. After dissolution with the aid of heat as explained above, the solution should be clear.

PRECAUTIONS

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION AND STORAGE

Use only clear, unhemolyzed serum, separated from the clot as soon as possible after drawing of the blood. Do not use plasma. Acid phosphatase at the normal pH of serum is very labile. To stabilize the enzyme proceed as follows: Immediately after separation of the serum from the clot, add 20 µL of the Acetate Buffer provided to each mL of sample. Mix. Store in refrigerator. The enzymatic activity in the sample will be stable for seven days.

INTERFERING SUBSTANCES

It has been reported (6) that high levels of bilirubin in serum inhibit acid phosphatase activity as determined by this procedure. Oxalates and fluoride inhibit the enzyme, while heparin and EDTA will cause turbidity. Do not use these anticoagulants.

Young (7) has published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays including that for acid phosphatase.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer or colorimeter capable of measuring absorbance at 405 nm.
2. Cuvettes, with a 1 cm light path.
3. Constant temperature incubator set at 30 °C. Temperature must be closely controlled during assay and spectrophoto-meter cells should be thermostated.
4. Distilled or deionized water.
5. Pipettes to measure water, reagent(s) and samples.

MATERIALS PROVIDED

1. Acid phosphatase reagent in dry powder form.
2. L-Tartrate in dry powder form.
3. Acetate buffer in solution.

TEST PROCEDURE

Immediately after separation of the serum from the clot, stabilize the acid phosphatase by adding to each mL of serum 20 µL of Acetate Buffer. Mix and store in refrigerator until the assay is performed.

	Wavelength:	405 nm
	Temperature:	30 °C
A.	Total Acid Phosphatase:	
	In square cuvettes pipette:	
	Acid Phosphatase Reagent	2.0 mL
	Serum	0.2 mL

Mix. Incubate for 5 minutes at 30 °C. Then follow the reaction at 30 °C, taking readings every minute, for 5 minutes. Deter-mine the ΔA/minute.

B.	Non-Prostatic Acid Phosphatase:	
	In square cuvettes pipette:	
	Acid Phosphatase Reagent	2.0 mL
	L-Tartrate Solution	0.02 mL
	Mix. Then add:	
	Serum	0.2 mL

Mix. Incubate for 5 minutes at 30 °C. Then follow the reaction at 30 °C, taking readings every minute, for 5 minutes. Deter-mine the ΔA/minute.

C. Prostatic Acid Phosphatase:

It is obtained by subtracting the results of the non-prostatic acid phosphatase assay (B) from those of the total acid phosphatase (A).

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QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for acid phosphatase activity may be used. Cliniqa Assayed Control Serum, Level 1 (R83082) and Level 2 (R83083) are recommended for this purpose.

CALCULATIONS

A. Total Acid Phosphatase

$$U/L = \Delta A/min. \times \frac{2.2 \times 10^6}{12.9 \times 10^3 \times 1 \times 0.2} = \Delta A/min. \times 853$$

B. Non-Prostatic Acid Phosphatase

$$U/L = \Delta A/min. \times \frac{2.22 \times 10^6}{12.9 \times 10^3 \times 1 \times 0.2} = \Delta A/min. \times 860$$

C. Prostatic Acid Phosphatase

A (U/L) - B (U/L) = Prostatic Acid Phosphatase (U/L)

Where: 2.2 and 2.22 = volume of assays

12.9 = mM extinction coefficient of diazo dye

1 = light path in cm

0.2 = volume of sample

Sample Calculation:

ΔA/min. Total acid phosphatase = 0.010

ΔA/min. Non-prostatic acid phosphatase = 0.005

Total acid phosphatase = 0.010 x 853 = 8.5 U/L

Non-prostatic acid phosphatase = 0.005 x 860 = 4.3 U/L

Prostatic acid phosphatase = 8.5 - 4.3 = 4.2 U/L

LIMITATIONS OF THE PROCEDURE

1. The assay can be performed at temperatures different from 30 °C, such as 25 °C or 37 °C. The activity will vary with changes in temperature, but the calculations will remain the same. Expected values will, however, be different.
2. If the ΔA/min. is greater than 0.041 at 30 °C (activity greater than 35 U/L), dilute the sample with nine volumes of physiological saline (sodium chloride: 150 mmol/L in water) and repeat the assay. Multiply the results by 10.

REAGENT PERFORMANCE

1. Linearity: The assay is linear to 35 U/L at 30 °C. Samples giving higher results should be diluted with 9 volumes of physiological saline and the assay should be repeated in this diluted sample. Multiply the results by 10.
2. Correlation: Employing as a reference a commercial reagent (Gilford) with the same formulation, using 56 serum samples (some of which were spiked with Lee Scientific prostatic acid phosphatase), ranging in value between 3.8 U/L to 105 U/L, the following correlations were obtained:

	Acid Phosphatase	
	Total	Prostatic
Correlation Coefficient	0.999	0.999
Regression Equation	y = 0.987 x + 0.195	y = 0.980 x + 0.316

3. Precision:

	Within Run			
	Total acid phosphatase		Prostatic acid phosphatase	
Mean U/L	6.1	23.9	5.9	22.7
SD	0.19	0.5	0.32	0.54
CV (%)	3.1	2.1	5.4	2.4
N	28	28	28	28

	Run to Run		
Mean U/L	2.1	7.7	19.9
SD	0.45	0.23	0.32
CV (%)	21	3	2.1
N	30	28	26

REFERENCE RANGES

The reference ranges for total serum acid phosphatase are:

30 °C 0 to 4.5 U/L

37 °C 0 to 5.1 U/L;

for prostatic acid phosphatase:

30 °C 0 to 0.8 U/L

37 °C 0 to 1 U/L

It is suggested that each laboratory establish its own reference range.

REFERENCES

1. Babson, A.L., Read, RA. and Phillips, G.E., *Am. J. Clin Path.* 32, 83, 1959.
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6. Shaw, L.M., Brummund, W. and Dozio, R.J., *Am. J. Clin. Path.,* 68, 57, 1977.
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IVD **For in vitro diagnostic use**

See package insert for proper use

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RE-ORDER INFORMATION
Acid Phosphatase Reagent

Catalog No.
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Catalog No.
REF R85128

Made in the USA

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